Controls with and without chondroplasty were prepared for both groups. Media was changed every two days and group 2 media was supplemented with either IgF-1 or JNK-II. Eight 1mm slices from representative condyles were cut with a precision saw. Fours slice each were used for the cell viability and depth of penetration assay.
Results: Group 1: IgF-1 (50ng/ml) showed a significant improvement (p=0.03) in PG on Day 2. JNK-II (25uM) displayed improved PG without significance (p=0.06) on Day 2.
Group 2: IgF-1 (25ng/ml) significantly enhanced PG on Day 3 (p=0.03). IgF-1 (50ng/ml) significantly inhibited PG on Day 1, 3 and 7 while JNK-II (25uM) significantly inhibited PG on Day 1. The average depth of penetration for the representative cartilages slice was 99um while the depth of cell death averaged 151um.
Conclusions: The data suggest that a higher IgF-1 concentration is required to elicit the cytoprotective effect if administered prior to chondroplasty and a lower concentration must be used if IgF-1 is administered after chondroplasty. At higher concentrations (post-chondroplasty), IgF-1 and JNK-II inhibited proteoglycan synthesis. This suggests that a threshold concentration is needed to elicit the cytoprotective effect but higher concentrations inhibit PG. The mechanism of how these agents work in protecting cells needs further elucidation. This was an in vitro study which contains inherent weakness of not having synovial factors in the mileu. Bovine cartilage was used in this experiment instead of human osteoarthritic or cadaver cartilage due to availability and tissue quality. Human osteoarthritic chondrocytes have the potential to react differently to the cytoprotective agents and thus must be evaluated. Young bovine cartilage was used since chondrocytes proliferate much faster in young animals and may also have a greater response to cytoprotective agent than older tissue. The superficial zone of chondrocytes (within 100um of the articular surface) has the highest level of cell proliferation. Since the depth of penetration in our experiment averaged 99um, most active chondrocytes have been removed by chondroplasty. These data support the hypothesis that pre or post treatment of articular cartilage with IgF-1 increases metabolic activity and provides cytoprotection when performing chondroplasty under these conditions.

P189

MOUSE CARTILAGE UNDER COMPRESSION: INDUCTION OF NUCLEAR FACTOR-kB AND EXTRACELLULAR SIGNAL REGULATED KINASE1/2 ACTIVITY AND MODULATION BY AVOCADO/SOYBEAN UNSAPONIFIABLES

O. Gabay, M. Gosset, A. Levy, C. Salvat, A. Pigenet, C. Jacques, F. Berenbaum
UMR 7079 CNRS/Paris VI, Paris, France

Purpose: We studied the main intracellular signalling pathways known to be involved in the prodegradative process of matrix cartilage (MAP kinases and NF-κB) in chondrocytes stimulated with the proinflammatory cytokine interleukin-1 beta (IL-1β) or in cartilage explants submitted to a mechanical stress (1MPa, 0,5 Hz). Moreover, we studied whether Avocado-Soybean Unsaponifiables (ASU), a common drug used in Europe for symptoms in osteoarthritis (OA), could modulate these intracellular signalling pathways.
Methods: Mouse costal chondrocytes in monolayer primary culture stimulated with IL-1β (10ng/ml) or mouse costal cartilage explants under mechanical stress (MS) were used in this study. The chondrocytes or explants were incubated in presence or absence of ASU (10μg/ml) NF-κB pathway was assessed by IκBα expression, by nuclear translocation of NF-κB using p65 antibody, by Electrophoretic Mobility Shift Assay (EMSA), using p50 and p65 antibodies. MAP kinase (MAPK) pathways were assessed by using phospho-p38, ERK1/2 and SAP/JNK protein expression.
Results: I-kBα expression is decreased by 70% in compressed cartilage (after 2 hours of compression). I-kBα expression is also decreased by 72% in presence of IL-1β (as soon as 2 minutes after stimulation), in parallel with the translocation of the cytosolic p65 subunit to the nucleus. Moreover, the binding of the heterodimer p50/p65 to NF-κB responsive element is significantly increased after IL-1β treatment. Interestingly, ASU partially prevent ILβ-induced degradation of IκBα by 39% and MS-induced degradation of IκBα by 28%.
IL-1β-induced binding of p50/p65 is significantly inhibited in presence of ASU, in parallel with an inhibition of the translocation of p65 into the nucleus. Whereas the 3 MAPK p38, JNK and ERK1/2 were activated in presence of IL-1β, ASU inhibited specifically the ERK1/2 pathway by 34%. A same profile was observed in MS-activated chondrocytes.
Conclusions: We show here that, along with IL-1β, MS is also a strong trigger for NFκB and ERK 1/2 activation suggesting that these 2 pathways are mechanosensitive in chondrocytes. Moreover, our study shows that ASU inhibit NF-κB and ERK1/2 pathways.

P190

THERMOGRAVIMETRIC INVESTIGATION OF NORMAL AND DAMAGED HUMAN HYALINE CARTILAGE

G. Sohár[1], K. Tóth[1], E. Pallagi[2], P. Szabó-Révész[2]
[1]Department of Orthopaedics, University of Szeged, Szeged, Hungary, [2]Pharmaceutical Technology Department, University of Szeged, Szeged, Hungary

Purpose: The purpose of this study was to elucidated the importance of water content in contributing to disease progression and to establish the kinetic character of water loss effect of heating. Previously, water content has not been measured thermoanalyticaly in normal and degenerative human hyaline cartilage. Therefore a new thermogravimetric protocol had to be established before the detailed investigation could be performed. Most of the known changes in the extra cellular matrix in OA comes from animal models since human samples for investigation are not widely available for experiment. The specific causes of osteoarthritis are unknown, but are believed to be a result of both mechanical and molecular events in the affected joint. Thermogravimetry (TGA) is one of the oldest thermal analytical procedures and has been used extensively in the study of polymeric systems.
Methods: During arthroplasty procedures performed at the Orthopaedic Department, University of Szeged, Hungary, degenerative human hyaline cartilage was obtained from 28 hip and normal cartilage from 7 knee. The samples were taken under sterile conditions, and excess bone was removed. Preoperatively the diagnosis of the patient was established on basis of the patient history, clinical signs and radiological findings. The state of the hyaline cartilage was determined intraoperatively. 35 samples were collected. Based on the patient diagnosis, seven samples were analyzed as normal hyaline cartilage, 12 were obtained from patients with femoral head necrosis, and 16 were collected from osteoarthritic cartilage. The thermogravimetrical analysis was performed with the use of a MOM Derivatograph (MOM, Budapest, Hungary), and the TG, DTG and DTA curves were determined.
Results: It was found, that the total water content of intact (healthy) cartilage was 80,79% (SD: 7.09%), of necrotic femoral head was 87.80% (SD: 8.06%), of the osteoarthritic samples was 86.71% (SD: 7.84%). To remove the cartilage extra cellular water content 52.33 (SD: 6.68) kJ/M energy was needed in normal samples, in aseptic femoral head necrosis needed 70.25 kJ/M